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(54) Title: METHOD FOR MAKING AUTOLOGOUS FIBRIN SEALANT

(57) Abstract

A method of producing a fibrin sealant. Platelet rich blood plasma and recombinant thromboplastin are mixed to effect the formation of fibrin sealant.

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METHOD FOR MAKING AUTOLOGOUS FIBRIN SEALANT

Description

Technical Field

The present invention relates to the preparation of fibrin based sealants.

Background Art

The preparation and use of fibrin based sealants is becoming more prevalent in medical practice. This is due to the biocompatibility of such sealants.

Biocompatibility has some significant issues, however, when the present methods of forming the sealants are examined. The most common method is to use what is known as bovine thrombin preparations. These type of preparations have been approved for medical use for several years; however, recent findings suggest that there are significant problems associated with their use. These problems include: 1) the risk of transmission of bovine spongiform encephalitis, and 2) the development of immune responses to the thrombin and contaminants in the thrombin which cause the development of autoimmune antibodies to various human coagulation factors. This results in patients who develop pseudohemophilia and are at increased risk for developing severe bleeding problems.

Human recombinant thromboplastin is presently available as a diagnostic reagent for use in performing various coagulation assays.

Fibrin sealants are made from several different types of starting materials, including: 1) citrated plasma, 2) concentrated citrated plasma, 3) platelet rich citrated plasma, 4) cryoprecipitates, and 5) purified plasma fractions which contain high quantities of fibrinogen. The coagulation of blood is a rather complex process. The primary reaction of producing a clot is caused by the action of thrombin on the fibrinogen molecule which converts fibrinogen to fibrin. Fibrin spontaneously polymerizes, forming a net-like structure. This structure is later solidified and enhanced by the actions of several other factors in blood, which factors are also generated by the action of thrombin. Most of the factors found in the blood are in an inactive form. Thrombin has an inactive form, prothrombin. Thus at some point there must be a trigger for the initiation of blood clotting. One of these mechanisms is thromboplastin. When blood or blood plasma is exposed to thromboplastin, it triggers the activation of these factors which leads to the generation of thrombin which in turn converts fibrinogen to fibrin.

Disclosure of Invention

It is the principal object of the present invention to provide an improved method of producing fibrin sealants or adhesives.

It is a further object of the present invention to provide an improved method of the foregoing character for producing fibrin sealants or adhesives wherein the risk of transmission of bovine disease and virile human disease which would be associated with the use of human thrombins purified from heterologous sources is substantially reduced or eliminated.

The present invention is embodied in a method for making a fibrin sealant adhesive or glue which does not introduce either immunologic or viral concerns. To this end, fibrin sealant is produced by utilizing human recombinant thromboplastin.

Best Mode for Carrying out the Invention

In accordance with the foregoing objects, the present invention is embodied in a method wherein human recombinant thromboplastin is mixed directly with the precursor of the fibrin sealant such as blood plasma, platelet rich blood plasma, concentrated blood plasma or cryoprecipitate. Alternatively, human recombinant thromboplastin is utilized to generate thrombin in a small aliquot of plasma or the supernatant from a cryoprecipitation, and then the thrombin thereby generated is combined with the precursor of the fibrin sealant. Both procedures produce a fibrin sealant which can then be used in the conventional manner.

METHOD 1

Fibrin Sealant Source: Platelet Rich Plasma

The platelet rich plasma is collected into a 1:9 volume of 3.8% sodium citrate. Low speed centrifugation leads to the production of the platelet rich plasma. To form the sealant, the platelet rich plasma is mixed with recombinant thromboplastin in a suitable container and sufficient calcium chloride is added to neutralize the citrate used as the anticoagulant. The ratios of recombinant thromboplastin, calcium, and platelet rich plasma are preferably determined in small test tubes. The desired result is to effect the formation of a fibrin sealant gel in one to two minutes after the combination of the above agents.

METHOD 2

Fibrin Sealant Source: Platelet Rich Plasma

Plasma Source for Thrombin: Prepared by High Speed Centrifugation

Blood plasma, citrated as described above, is mixed with recombinant thromboplastin and calcium. The resulting clot is agitated to break up the clot. The supernatant fluid, which contains thrombin, is separated by centrifugation. The thrombin is then used as in Method 1 to generate a fibrin sealant from the platelet rich plasma.

Claims

1. A methd of producing a fibrin sealant comprising mixing platelet rich blood plasma and recombinant thromboplastic to effect the formation of fibrin sealant.

2. A method of producing a fibrin sealant comprising mixing citrated blood plasma, recombinant thromboplastin and calcium, separating thrombin from said mixture, and adding said thrombin to platelet rich plasma to generate fibrin sealant.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08472

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L25/00 A61K35/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US SUZUKI M. ET AL.: "CLINICAL APPLICATION OF THE FIBRIN ADHESIVE" XP002040697 see abstract & OTOLARYNGOLOGY, vol. 56, no. 11, 1984, TOKYO, pages 949-953, ---	1,2
E	WO 97 29792 A (COHESION CORP ;SIERRA DAVID H (US)) 21 August 1997 see the whole document --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 September 1997	30-09-1997

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 443 724 A (BAXTER INT) 28 August 1991 see column 1, line 41 - line 54 see column 5, line 51 - line 58; claims	1,2
A	FR 2 696 095 A (INOTEB) 1 April 1994 see claims; examples	1,2

INTERNATIONAL SEARCH REPORT

[illegible]ation on patent family members

Int[illegible]ional Application No
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